DURATION AND FREQUENCY OF WING BEAT IN THE AGING HOUSE FLY, MUSCA DOMESTICA L.[1]

MORRIS ROCKSTEIN AND PREM LATA BHATNAGAR

Department of Physiology, University of Miami School of Medicine, Coral Gables, Florida 33134

In a long-range study of aging in the house fly, Rockstein and his co-workers (Rockstein, 1956, 1957; Rockstein and Brandt, 1963; Rockstein and Gutfreund, 1961) have previously noted that abrading and ultimate loss of wings, especially in the male house fly, is preceded and then accompanied by the failure or decline of specific intracellular biochemical components (enzymes, coenzymes, etc.) of the thoracic flight muscle, which are directly or indirectly concerned with the energizing of the contraction of flight muscle. Corresponding microanatomical changes were similarly reported by Rockstein and Bhatnagar (1965) in describing the age-related distribution of number and size of giant mitochondria of maturing and senescent male and female house flies.

The study on which this report is based was undertaken in order to establish more precisely the age-related, quantitative changes in flight ability, *i.e.*, the wing beat frequency and duration of flight, for male and female house flies from emergence to senility.

Materials and Methods

A long-inbred (NAIDM) strain of *Musca domestica* L., maintained in our laboratory at 80° F. and 45% R.H., was used in this study. The experimental population was obtained by allowing 4- to 5-day-old females to lay eggs on a standardized artificial medium described earlier (Rockstein, 1957). From the time of emergence and during the course of the entire experiment, all flies were allowed to feed *ad libitum* on sucrose, twice daily, for a period of one hour each feeding; such flies were considered to be fully satiated as far as their carbohydrate requirements were concerned. A continuous supply of water was provided throughout the period of study.

For the study of wing beat frequency (WBF), flies of known age, immediately after having been previously fed on sucrose for an hour, were anesthetized under carbon dioxide, sexed, and mounted (attached individually in the dorsal midthoracic region with Duco® cement) onto thin, inverted "J"-shaped copper wire supports, which have been set in fine holes drilled in a wooden block 6″ × 1.5″. Removal of tarsi or of any substratum, essential for the initiation and sustenance of flight in the case of *Phormia* (Friedman, 1959; Clegg and Evans, 1961) or of *Drosophila* (Williams *et al.*, 1943), was found to be quite unnecessary for initiating or maintaining flight in the house fly, which normally flies spontaneously when so sup-

[1] Supported in part by funds from the United States Public Health Service, Research Grant No. HD 00571 from the National Institute of Child Health and Human Development.

ported. This total process of anesthetizing and mounting takes less than 5 minutes and, if normal, the flies begin to fly spontaneously within two to three minutes after having been mounted and after recovery from CO_2 anesthesia. In fact, those flies which did not fly spontaneously would not do so even upon stimulation of their tarsi or of the ventral surfaces of the abdomen. All phases of the experiments were conducted under constant conditions of temperature, humidity and light as previously described by Rockstein (1956).

Wing beat frequency was measured by means of a Xenon Stroboscope (Cenco), with the mounted flies placed about 6 inches from the emission tube and observations made at 5-minute intervals and expressed as a percentage of the initial rate (Clegg and Evans, 1961). In each case, such observations were continued until 90% or more of the flies could no longer fly. Such experiments were repeated over a period of four generations.

TABLE I

Wing beat frequency and duration of flight of the female house fly as a function of age

Age (days)	No. of specimens	Initial WBF ±S.E.	Flight duration (in minutes)
1	16	8463 ± 109	500
2	17	9584 ± 151	475
4	22	9598 ± 129	455
5	10	9838 ± 189	465
6	25	9852 ± 136	470
7	29	9918 ± 111	440
10	28	9965 ± 164	398
15	33	9869 ± 166	225
19	19	9857 ± 207	105
22	22	9855 ± 150	110

RESULTS AND DISCUSSION

Females

Table I shows that the average WBF of a one-day-old female fly is 8500 beats per minute (bpm) and that this increases to 9600 by the second day and reaches a maximum of approximately 10,000 bpm by the seventh day; thereafter, there is very little change in WBF up to the third week, with perhaps a slight (if at all significant) diminution in WBF at the beginning of the third week and into the last day of adult life on which such observations could be made.

The time course of WBF of a one-day-old female is represented in Figure 1. For the first 155 minutes, the flies fly at a maximum WBF and thereafter they show a gradual decline. Even after 500 minutes of continuous flight, however, the flies show speeds as high as 90% of the initial WBF. Two-day-old female flies, with a WBF of 9600 beats per minute, show a constant WBF flight pattern up to about 250 minutes (Fig. 2), when all of the flies fly at about 95% of the initial WBF. However, during the last 200 minutes of flight, the WBF of such two-day-old females declines gradually to a low of less than 80% of the original. For female flies up to 10 days of age, the WBF and the time course of flight patterns are quite similar to those of younger, two-day-old females. However, only

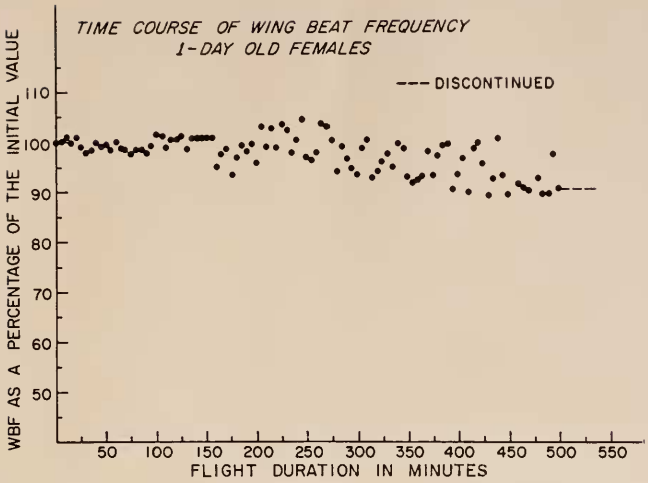

FIGURE 1. Time course of wing beat frequency in one-day-old female house flies.

after 12 days, when the WBF is still at its peak level, does the time course of flight change appreciably (Fig. 3), with a more rapid decline within 310 minutes to a WBF minimum. Finally, in 19-day-old females (Fig. 4), there is a rapid decline to a minimum in WBF in a little over 100 minutes.

Thus, the most conspicuous manifestation of senescence in flight function is the inability of very old flies to sustain flight for any extended periods of time. To recapitulate (as can be seen from Table I), there is a slow, steady, day-to-day decline in the duration of flight, so that by the third week, the ability of the female house fly to maintain flight for long periods of time is reduced markedly from 500 minutes in one-day-old to 110 minutes in 22-day-old female flies. This represents a decline in the ability of aging females to maintain flight to about 50% of the

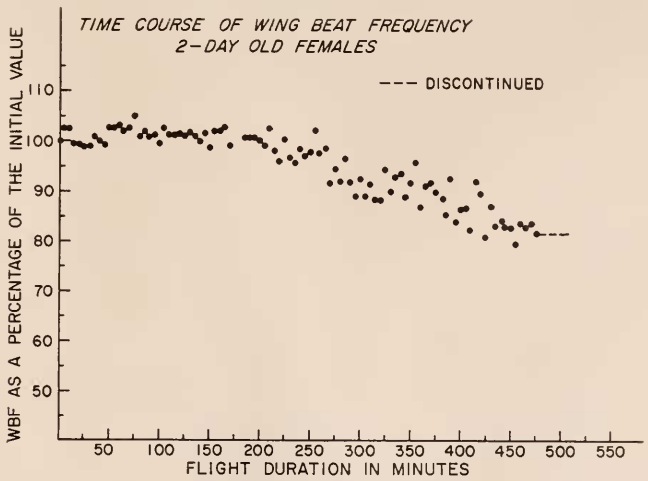

FIGURE 2. Time course of wing beat frequency in two-day-old female house flies.

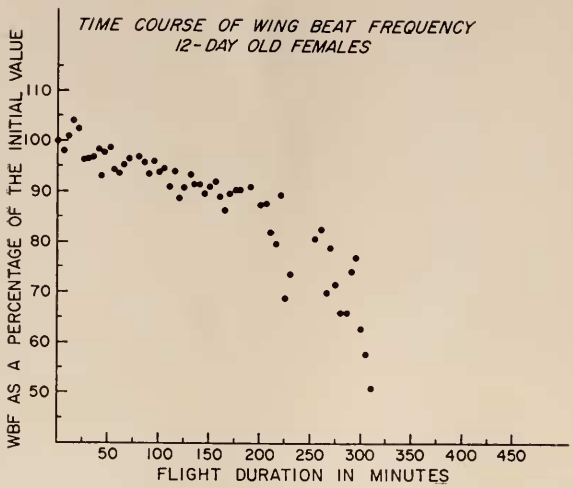

FIGURE 3. Time course of wing beat frequency in 12-day-old female house flies.

maximum level of flight duration at two weeks of age and to 20% of the initial maximum by the third week of adult life.

Males

As regards age-related failure in flight ability of the senescent male house fly, it should be emphasized that this particular study was carried out under special conditions of maintenance of all adult flies (*i.e.*, feeding them sucrose alone). Under such conditions, the rate of dying is much accelerated, *i.e.*, 60% of the males were dead by the 7th day, and of the remaining males at this age, only a few retained their wings (see Rockstein, 1956).

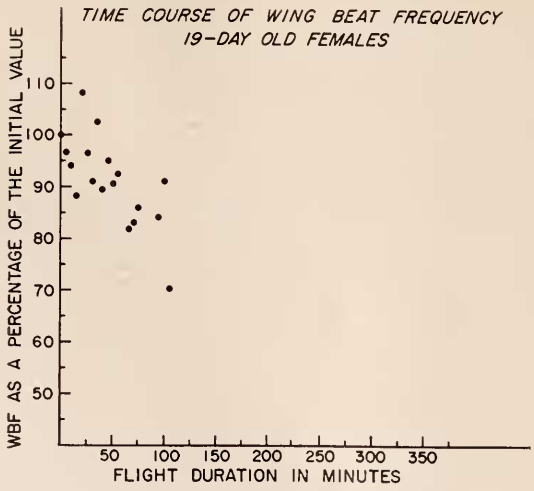

FIGURE 4. Time course of wing beat frequency in 19-day-old female house flies.

TABLE II

Wing beat frequency and duration of flight of the male house fly as a function of age

Age (days)	No. of specimens	Initial WBF ± S.E.	Flight duration (in minutes)
1	19	8639 ± 141	420
2	21	9328 ± 109	365
4	28	9710 ± 193	265
5	13	9698 ± 183	220
6	26	9733 ± 123	180
7	17	9801 ± 138	135
8	9	9821 ± 163	125
9	8	9700 ± 183	63

Moreover, under the above-mentioned conditions, 60% to 70% of the male population in the fly colony died within five to six days of eclosion and, out of the 30% to 40% remaining survivors which could be studied for flight ability, only a few retained intact wings, even as early as the end of the first week of imaginal life.

From Table II, it can be seen that one-day-old males fly at about 8600 wing beats per minute, which compares favorably with that for females of the same age (see Table I, above). This increases to about 9300 wing beats per minute during the following 24 hours. Maximum WBF of about 9700 to 9800 bpm is reached within four days after emergence and this remains unchanged through the ninth day of adult life, following which time no winged flies were available.

Figure 5 shows WBF as a function of flight duration in the one-day-old male house fly. The time pattern of the WBF of such young flies appears to be very similar to that of two-day-old female house flies (Fig. 2), *i.e.*, the WBF declines by about 18% by the end of 420 minutes, at which time the majority of the flies have stopped flying.

By the fifth day after emergence, male flies show a sharp decline in their WBF (Fig. 6) with time, *i.e.*, from the onset to the termination of flight, with the majority

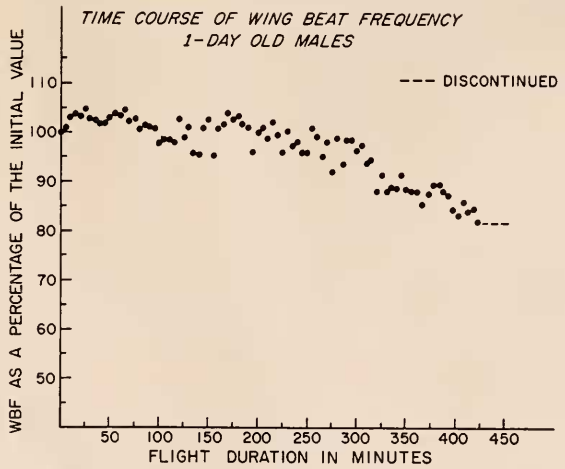

FIGURE 5. Time course of wing beat frequency in one-day-old male house flies.

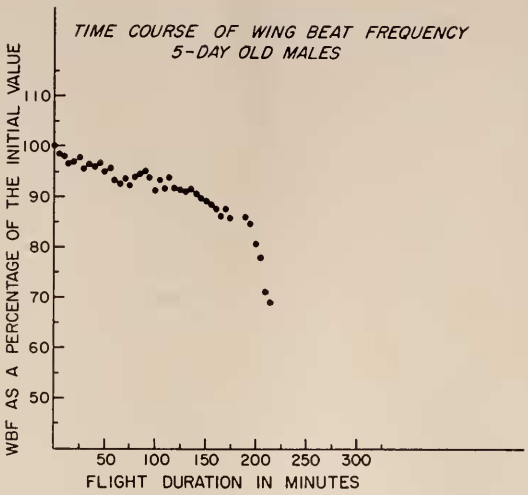

FIGURE 6. Time course of wing beat frequency in 5-day-old male house flies.

of flies showing a decline of about 30% within 215 minutes. The rapidity of such decline becomes more pronounced with age, so that seven-day-old males (Fig. 7) also show a decline by about 30% of WBF, but at 130 minutes after the initiation of flight.

It is quite apparent that the detailed data from these experiments confirm the more gross manifestations of senescence in flight ability, *i.e.*, the gradually increasing rate of failure of wing retention (male flies especially) previously observed by Rockstein (1956) and by Rockstein and Brandt (1963).

It would therefore appear from these data that the onset of decline in the motor function of flight in this holometabolous species clearly begins, as might be expected,

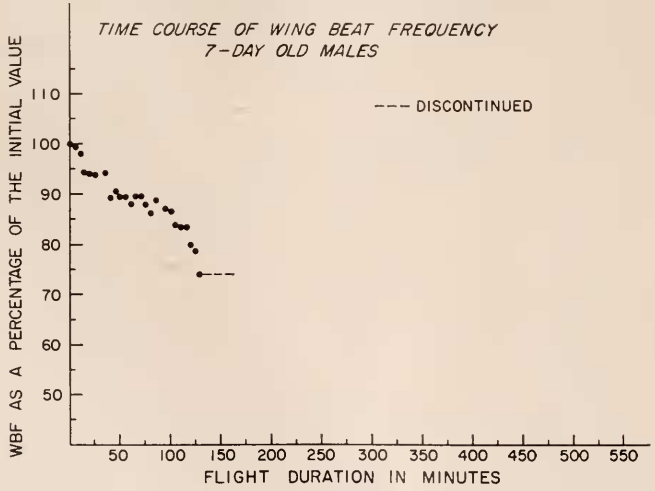

FIGURE 7. Time course of wing beat frequency in 7-day-old male house flies.

shortly after emergence, when the insect is fully mature and all of its tissues are essentially post-mitotic (with the exception of sexual maturation).

However, Williams *et al.* (1943) found this not to be true in *Drosophila funebris,* where both flight duration and wing beat frequency reach a peak by about 7 days and then drop off rapidly to a minimum by 35 days of adult life. However, no distinction as to relative flight ability of male and female *Drosophila* (either as regards WBF or duration of flight) was made by these authors, a distinction which both our present study and others have indicated may be a significant factor in all species studied.

In the two other important reports in which flight ability was studied (both in *Phormia regina*), unfortunately only WBF, with no distinction as to sex, was determined for aging adult blow flies by Levenbook and Williams (1956) and without regard to sex or age by Clegg and Evans (1961). It is therefore difficult to attempt comparisons between data for this present study and those in the relatively few past studies of this kind.

As for the trends observed in Tables I and II for wing beat frequency, values of about 10,000 beats per minute in mature males and female house flies resemble closely those reported both by Levenbook and Williams (1956), and by Clegg and Evans (1961) for *Phormia regina.* It is clear, however, that the parameter of duration of flight is much more significant, from the standpoint of aging, and that this parameter must also be measured separately for male and female adults, at least as far as the common house fly, *Musca domestica,* is concerned.

Finally, from the standpoint of *senescence* of flight ability, the steady diminution in the capacity of male house flies to sustain the original high levels of flight intensity (WBF) for extended periods of time confirms previously obtained quantitative, time-related data for wing loss as such, and decline in ATP-ase, alpha-glycerophosphate dehydrogenase and acid phosphatase activity, both in the sarcosomes and in the extrasarcosomal elements of the flight muscle (Rockstein, 1956; Rockstein and Brandt, 1963).

Summary

1. The age-related changes in wing beat frequency and duration of flight were studied in senescent male and female NAIDM house flies.

2. The average wing beat frequency increases to a maximum by the fifth day in female and by the fourth day in male house flies.

3. Duration of flight shows a steady, day-to-day decline with age. In females, this falls from 500 minutes for one-day-old to 110 minutes for 22-day-old flies. For males, this drop is more striking, with duration of flight falling from 420 minutes in one-day-old to 63 minutes for nine-day-old males.

4. These findings correspond to and confirm quantitatively previously reported data for wing loss and similar age-related changes in enzyme and coenzyme content in the flight muscle of senescent house flies.

LITERATURE CITED

Clegg, J. S., and D. R. Evans, 1961. The physiology of blood trehalose and its function during flight in the blowfly. *J. Exp. Biol.,* 38: 771–792.

Friedman, S., 1959. Sustained flight in *Phormia* (by a new method) and its effect on blood pH. *J. Ins. Physiol.,* 3: 118–119.

Levenbook, L., and C. M. Williams, 1956. Mitochondria in the flight muscles of insects. III. Mitochondrial cytochrome *c* in relation to the aging and wing beat frequency of flies. *J. Gen. Physiol.*, **39**: 497–512.

Rockstein, M., 1956. Some biochemical aspects of aging in insects. *J. Gerontol.*, **11**: 282–285.

Rockstein, M., 1957. Longevity of male and female house flies. *J. Gerontol.*, **12**: 253–256.

Rockstein, M., and P. L. Bhatnagar, 1965. Age changes in size and number of the giant mitochondria in the flight muscle of the common house fly (*Musca domestica* L.). *J. Ins. Physiol.*, **11**: 481–491.

Rockstein, M., and K. Brandt, 1963. Enzyme changes in flight muscle correlated with aging and flight ability in the male house fly. *Science*, **139**: 1049–1051.

Rockstein, M., and D. E. Gutfreund, 1961. Age changes in adenine nucleotides in flight muscle of male house fly. *Science*, **133**: 1476–1477.

Williams, C. M., L. A. Barness and W. H. Sawyer, 1943. Utilization of glycogen by flies during flight and some aspects of the physiological ageing of *Drosophila*. *Biol. Bull.*, **84**: 263–272.